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Growth of Cotton under Chronic Ozone Stress at Two Levels of Soil Moisture

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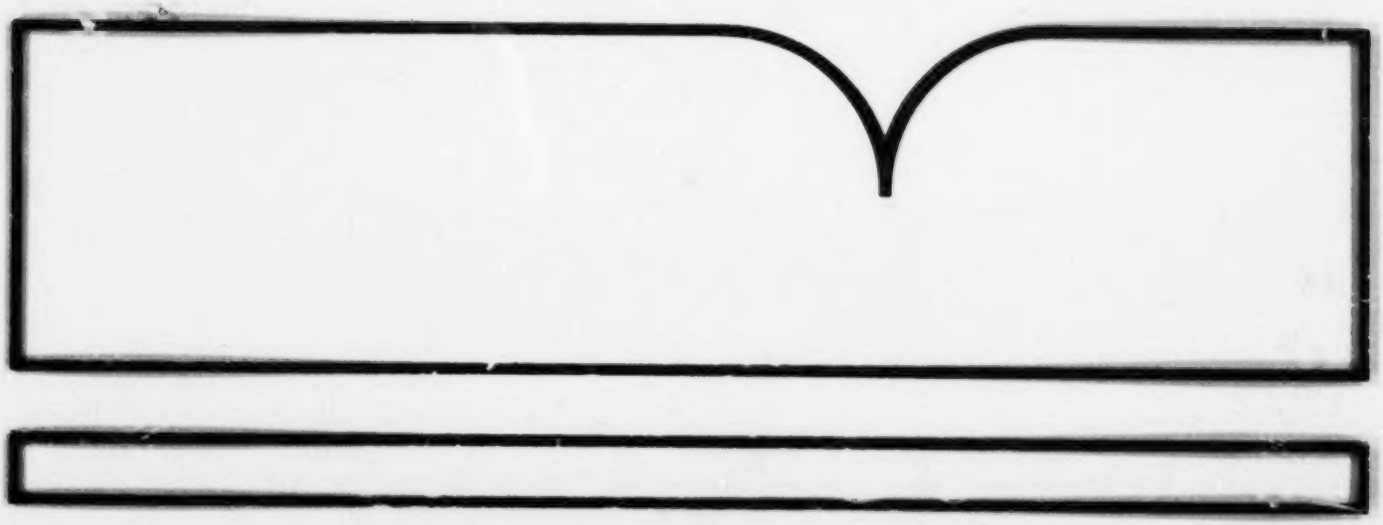

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16. ABSTRACT

Recent field research has shown that ambient concentrations of ozone(O_3) have the potential to reduce yield of a number of economically important crop species. Less emphasis has been placed on characterization of the effects of O_3 on growth of the total plant that ultimately determines yield. In this study, we measured season-long growth of field-grown cotton that was exposed to a range of O_3 concentrations at two levels of soil moisture, well-watered (WW) and water-stressed(WS). The cotton was exposed for 12 h d^{-1} throughout the growing season in open-top chambers to five O_3 concentrations, that ranged from 0.020 to 0.074 uL L^{-1}(seasonal mean 12 h d^{-1} concentration). Three cotton plants were sampled from each chamber at 2-to 3-wk intervals during the season, and biomass and growth measurements were made on all major plant parts. Ozone stress reduced leaf and root biomass in particular, while stem tissues were less affected. Partitioning of biomass among plant tissues was affected by O_3 at certain times during the season. Leaf area duration (LAD) was reduced by O_3 in both WW and WS treatments, but changes in the yield/LAD ratio and net assimilation rate indicated that reduced efficiency of leaves also was responsible for yield reductions in the WW plots. Water stress caused fewer significant effects overall than did O_3, although it appeared to reduce the growth of most plant parts, especially at low O_3 concentrations. There were no significant O_3 x water interactions for the growth variables measured.

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Growth of Cotton under Chronic Ozone Stress at Two Levels of Soil Moisture

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ABSTRACT

Recent field research has shown that ambient concentrations of ozone (O_3) have the potential to reduce yield of a number of economically important crop species. Soil water levels have been shown sometimes to modify the extent of the reduction. The primary emphasis of most of these studies has been on the plant component that represents economic yield. Less emphasis has been placed on characterization of the effects of O_3 on growth of the total plant that ultimately determines yield. In this study, we measured season-long growth of field-grown cotton (*Gossypium hirsutum* L. 'McNair-235') that was exposed to a range of O_3 concentrations at two levels of soil moisture, well-watered (WW) and water-stressed (WS). The cotton was exposed for 12 h d^{-1} throughout the growing season in open-top chambers to five O_3 concentrations that ranged from 0.020 to 0.074 $\mu L\ L^{-1}$ (seasonal mean 12 h d^{-1} concentration). Three cotton plants were sampled from each chamber at 2- to 3- wk intervals during the season, and biomass and growth measurements were made on all major plant parts. Ozone stress reduced leaf and root biomass in particular, while stem tissues were less affected. Partitioning of biomass among plant tissues was affected by O_3 at certain times during the season. Leaf area duration (LAD) was reduced by O_3 in both WW and WS treatments, but changes in the yield/LAD ratio and net assimilation rate indicated that reduced efficiency of leaves also was responsible for yield reductions in the WW plots. Water stress caused fewer significant effects overall than did O_3, although it appeared to reduce the growth of most plant parts, especially at low O_3 concentrations. There were no significant O_3 × water interactions for the growth variables measured.

Ozone (O_3) and water stress are known to affect the yield of cotton. Experiments using open-top chambers to expose cotton to controlled levels of O_3 have shown that yield may be reduced from approximately 7 to 20% by ambient O_3 concentrations that occur in cotton-growing regions when soil moisture is adequate (Temple et al., 1985; Heagle et al., 1986a, 1988). Since soil water is often limiting during the growth of cotton, the effects of water stress and its potential interaction with O_3 stress have also been investigated. The effects of water stress and its interaction with O_3 stress appear to depend on climatic conditions. Temple et al. (1985) found a yield reduction due to water stress and a reduction of the O_3 effect by water stress during a hot and dry season. A repeat of the experiment during a cool and cloudy season indicated no significant effects of water stress and no modification of the O_3 effect by water stress. Heagle et al. (1988) found that moderate water stress (itself causing a 16% decrease in yield at low O_3 concentrations) reduced the severity of the O_3 effect on yield.

While a considerable amount is known concerning the extent to which O_3 influences crop yield, less is understood concerning its effect on growth and development, especially under field conditions. Evaluation of O_3 effects throughout growth and development should contribute to an understanding of the mechanisms through which the stress is expressed and thereby provide a better basis for evaluation of potential yield effects. Ozone effects on plant growth, development, and partitioning of biomass have been studied under controlled-environment conditions with a number of plant species (Miller, 1987). For example, in a greenhouse study with cotton, Oshima et al. (1979) found that O_3 reduced the dry weight of all plant parts, with the largest reductions occurring in roots and bolls. The effects were ascribed to reductions in leaf area, net assimilation rate, and a shift in the allocation of C.

The effects of water stress on growth, development, and biomass partitioning have been studied more extensively than for O_3. Water stress may reduce vegetative

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growth, increase senescence of leaves, and alter partitioning of biomass (e.g., Boyer, 1968; Taylor et al., 1983). With cotton, it is well documented that stem elongation and leaf growth are retarded by water stress in the field (Grimes et al., 1978; Marani and Levi, 1973; Wadleigh and Gauch, 1948). Top growth of cotton ceases when morning leaf water potential decreased to −0.5 to −0.7 MPa (Klepper et al., 1973) or when midday values reached −1.4 MPa (Marani et al., 1985).

Two studies have reported the combined effects of O_3 and water stress on growth, development, and biomass partitioning. Amundson et al. (1986) found that O_3 and water stress separately reduced the growth of soybean [*Glycine max* (L.) Merr.] grown in controlled-environment chambers, but neither caused changes in biomass partitioning. Water stress tended to reduce the damaging effects of O_3, probably by partially closing stomates and reducing gas exchange. Similarly, Flagler (1986) found in a 2-yr field study that O_3 tended to reduce the growth of most parts of soybean and that the O_3 effects were greatest in the treatments having adequate soil moisture. Biomass partitioning was affected by both stresses, but the results varied across years.

Additional research is needed to understand the effects of O_3 and water stress, and their combination, on crop growth and development under field conditions. The study reported here was part of a project to evaluate effects of O_3 and water stress on yield of an eastern cotton cultivar (McNair-235) using open-top field chambers (Heagle et al., 1988). The specific objectives of the study were to: (i) measure the effects of O_3 and water stress on the growth and development of cotton in the field; (ii) determine if water stress alters plant response to O_3; and (iii) relate the growth and development findings to the yield results of Heagle et al. (1988).

MATERIALS AND METHODS

PLANT GROWTH

Cotton (McNair 235) was planted on 29 May 1985 in a field located approximately 8 km south of Raleigh, NC. The soil consisted of about 30 cm of Norfolk loamy sand (fine-loamy, siliceous, thermic Typic Paleudult) overlaying an Appling sandy loam (clayey, kaolinitic, thermic typic Hapludult). The field was limed and fertilized according to soil test recommendations. Cotton was machine-planted in 1-m rows and additional rows were planted with a hand-pushed planter between the 1-m rows to give a final row spacing of 0.5 m. Twenty-four plots, each containing three 3-m rows (the size needed for open-top chambers), were chosen on 11 June based on uniformity of the plant stand and plant size. Plants were thinned to 11 plants m^{-1} of row on 11 June. Other aspects of plant culture such as weed and insect control are reported in Heagle et al. (1988).

OZONE TREATMENTS

Ozone treatments were administered by use of open-top chambers (Heagle et al., 1973), which were operated from 26 June through 30 October. The experiment was a completely randomized factorial with five O_3 levels and two soil moisture levels in open-top chambers with two replicates (plots) for each O_3-moisture combination. In addition, there were two replicates of an ambient air (AA) treatment (no chamber) for each moisture level, although these are not included in the data analyses. The five chamber treatments were charcoal-filtered air (CF), nonfiltered air without added O_3 (NF), and three NF treatments with O_3 added in different proportions of ambient O_3, using dispensing and monitoring methods as described previously (Heagle et al., 1986b). The three proportional treatments were approximately 1.2, 1.4, and 1.7 times the NF O_3 concentration (NF × 1.2, NF × 1.4, and NF × 1.7). Ozone additions were made from 800 to 2000 EST when the ambient O_3 concentration exceeded 0.03 $\mu L\ L^{-1}$. The daily O_3 additions began on 28 June, when plants were approximately 20 cm tall and had four expanded secondary leaves, and continued throughout the period of plant sampling.

SOIL MOISTURE TREATMENTS

The two soil moisture treatments were established during periods of low rainfall by differential irrigation of the well-watered (WW) and water-stressed (WS) plots. Soil moisture was measured in all plots with a neutron probe (Model 503 DR, Campbell Pacific Nuclear, Pacheco, CA).[1] The soil moisture criteria used to determine when irrigation occurred were soil matric potentials at 23-cm depth of −0.10 MPa and −0.30 MPa for the WW and WS plots, respectively. Irrigation consisted of approximately 2.5 cm of water applied to each chamber with drip tubing located at the base of plants in each row.

LEAF WATER POTENTIAL MEASUREMENTS

Leaf water potential of the third or fourth open leaf (counting down from the growing point) was measured at midday (1100 to 1300 EST) during stress cycles with thermocouple psychrometers by the dew point technique. Chambers similar to those described by Brown and Collins (1980) were used. The psychrometers were calibrated against salt solutions of known concentrations.

PLANT SAMPLING

The center row and the southern half of the other two rows were sampled in this study. The remaining row segments were used for final cotton yield determinations for a separate study. Five adjacent plants per plot were sampled on 28 June [30 d after planting (DAP)], 15 and 29 July (47 and 61 DAP), 12 August (75 DAP), 3 and 23 September (97 and 117 DAP), and 11 October (135 DAP). The first two samplings were from the center row and then alternated between the east and west row at each successive date. The center row was removed before canopy closure occurred. The plots were sampled in random order on each date between 1100 and 1500 h EST. The intact plants were removed by carefully extracting the roots from soil that had been loosened with a space. The majority of the taproot and the larger lateral roots were recovered, although some smaller fibrous roots were not. Two plants were left between each group of five plants as a border. This was necessary because the roots of adjacent plants were disturbed during plant removal. Three of the five plants sampled were randomly selected for determination of biomass of the individual tissues. Immediately after sampling, the three plants were separated into their component tissues, i.e., leaves, stem structures (main stem, branches, and petioles), root, and reproductive structures (squares, flowers, and bolls). The tissues were oven-dried to constant weight at 80 °C and weighed. The remaining two plants were used for analysis of tissue carbohydrates (data to be published separately).

[1] The use of trade names in this publication does not imply endorsement by the North Carolina Agric. Res. Serv. or the USDA of the products named, nor criticism of similar ones not mentioned.

STATISTICAL AND GROWTH ANALYSIS

To test for treatment-related effects over the course of the study, analyses of variance (ANOVA) were performed on the $\log_e$-transformed variables where individual plant samplings were treated as split-plots in time (Steel and Torrie, 1980). Ozone, soil moisture (water), and O_3 × water effects, as well as the interactions of those effects with sampling date, were tested using the appropriate error terms. Main effects of sampling date were not tested since there was no appropriate error term and it was assumed that the variables change with time. Split-plot analyses were run for water treatments both separately and combined, but the results are presented only for the combined data because little additional information was gained from the separate analyses. Analyses of the growth and biomass data were performed according to the procedures outlined by Hunt (1982). Growth curves were generated by fitting second order polynomials to the $\log_e$-transformed dependent variables. The SAS was used in all analyses (SAS Inst., 1985).

RESULTS AND DISCUSSION

Ozone Doses

During the period when O_3 was added (28 June–30 October) the ambient plot O_3 concentration (seasonal mean 12 h d^{-1}, 0800–2000 EST) averaged 0.044 $\mu L\ L^{-1}$. The concentrations in the chambered plots (combined for soil moisture treatments and replicates) were 0.022, 0.041, 0.051, 0.061, and 0.073 $\mu L\ L^{-1}$ for the CF, NF, NF × 1.2, NF × 1.4, and NF × 1.7 treatments, respectively. The highest ambient 1-h peak was 0.119 $\mu L\ L^{-1}$. The 1-h federal secondary ambient air quality standard (0.12 $\mu L\ L^{-1}$) was not exceeded in the NF plots but was in all three treatments receiving O_3 additions. See Heagle et al. (1988) for a more thorough description of the O_3 concentrations.

Soil and Leaf Water Potentials

The soil matric potential averaged −0.03 and −0.13 MPa over the 30 occasions that it was measured in the WW and WS plots, respectively. The values in the WW plots were less than the target minimum (−0.1 MPa) on one occasion (19 July). Midday leaf water potentials in the WW plots were greater than in the WS plots each time they were measured. The averages were −1.5 and −2.1 MPa on the days measurements were made in the WW and WS plots, respectively. Values were −2.0 MPa or less on five occasions in the WS plots but only once in the WW plots. Wilting in the WW plots was apparent only on 19 July, while wilting occurred in the WS plots during each of the five periods in which soil matric potential was < −0.25 MPa. See Heagle et al. (1988) for more detail on soil and plant water status.

Growth

Curves were fit to the growth and biomass data in order to examine treatment related trends over the course of the growing season. Plots of the predicted values (obtained from second order polynomial equations fit to the $\log_e$ of the data) illustrate the effects of O_3 and water stress on biomass and growth (Fig. 1 and 2). (Note that the curves are not shown for all O_3 treatments if two or more overlap, as described in the figure legends.) The statistical significance of the O_3 and water effects and their interactions with sample date (S) in the split-plot ANOVA over time are shown in Table 1. Ozone × water interactions were not significant for any reported variable and thus are not included in the table. Interactions of S with O_3 or water occurred for several variables and usually these reflect the development of the stress response as the season progressed. A three-way interaction (S × O_3 × water) occurred only for total reproductive dry weight (dry wt.) and this is not included in Table 1 since it appeared to be fortuitous.

Table 1. Summary of analysis of variance for ozone and water-stress effects on cotton growth variables treating the seven plant sample dates (S) as split-plots over time.‡

Variable	O_3	H_2O	S × O_3	S × H_2O
Total plant dry wt.	†	NS	NS	NS
Total leaf dry wt.	*	NS	**	**
Total leaf area	*	NS	**	**
No. of main-stem leaves	**	†	**	**
Main-stem leaf area	**	NS	**	**
Leaf size (area/leaf)	*	†	**	NS
Root dry wt.	**	NS	NS	NS
Total stem dry wt. (main-stem, branches, petioles)	NS§	NS	NS	NS
Total shoot dry wt. (all aboveground parts)	†	NS	NS	NS
No. of main stem nodes	NS	NS	NS	†
Height	NS	**	NS	**
Total reproductive dry wt. (flowers, squares, bolls)	†	NS	NS	NS
Boll dry wt.	*	†	NS	**
Shoot dry wt./root dry wt.	NS	NS	NS	NS
Total stem dry wt./root dry wt.	†	†	NS	NS
Specific leaf area (m^2/kg)	†	**	NS	NS
Leaf weight ratio (kg leaf/kg plant)	*	**	**	**
Leaf area ratio (m^2 leaf/kg plant)	†	*	**	**
Net assimilation rate ($g/m^2\ d^{-1}$)	*	NS	**	NS

*,**,† Significant at the 0.05, 0.01, and 0.10 probability levels, respectively.

‡ Analysis of $\log_e$-transformed data according to Steel and Torrie (1980). Ozone × water stress interactions were not significant and thus not included: S × O_3 × water interactions significant at $p < 0.05$ for reproductive dry wt.

§ NS = not significant.

The split-plot ANOVA over time for total (vegetative plus reproductive) plant dry wt. indicated that the O_3 effect was significant at $p < 0.10$ (Table 1). In the WW treatments, effects of O_3 on the total dry wt. of the plants became apparent by 60 to 80 DAP (Fig. 1a). In the WS treatments, obvious effects of O_3 occurred only at the highest concentration (Fig. 1b). Although water stress appeared to reduce total plant dry wt. at the lower O_3 concentrations, there was no significant water treatment effect.

Leaves were markedly affected by O_3 stress as shown by the growth curves for total leaf dry wt. and area (Fig. 1c–1f), and the statistical summary (Table 1). The leaf responses were less apparent in the WS plots than in the WW plots, with only the two highest O_3 concentrations causing reductions in the WS plots. The main-stem leaves (growth data not shown) were extensively affected by O_3 (Table 1). The WW plots had more main-stem leaves than the WS plots midway in the growing season, but this was reversed later in the season due to greater leaf drop due to O_3 stress in the WW plots. Leaf size (for total leaves)

also was reduced by both O_3 and water stress. Interactions of O_3 or water stress with sampling date occurred frequently for the leaf variables (Table 1). This reflects the development of the season.

Root dry wt. was significantly affected by O_3 (Table 1). The growth curves for root dry wt. indicate a reduction by O_3 starting about 50 to 60 DAP (Fig. 2a and 2b). In the WW plots, only the two highest O_3 concentrations caused consistent reductions, while in the WS plots the reductions appeared to be more consistent across treatments.

Stems were not as extensively affected by O_3 or water stress as leaves and roots. Stem tissue dry wt. (main stem, branches, and petioles) was not significantly affected by O_3 or water stress (Table 1), although it appears that the

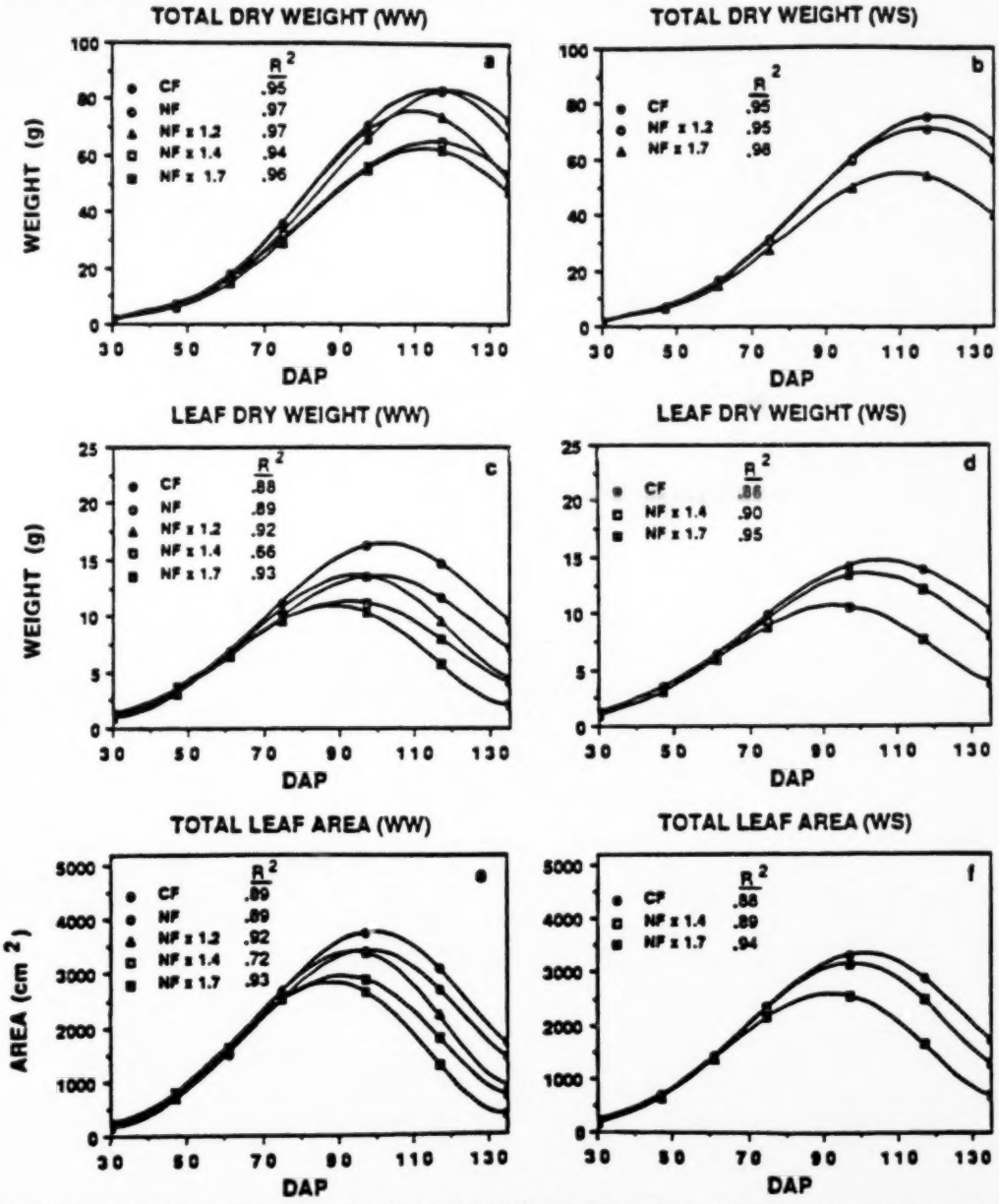

Fig. 1. Effects of chronic O_3 exposure on total dry wt. (*a* and *b*), total leaf dry wt. (*c* and *d*), and total leaf area (*e* and *f*) per plant of McNair-235 cotton under well-watered (WW) and water-stressed (WS) conditions. Ozone exposures started on 30 DAP and continued through the last sampling date (135 DAP). Growth curves are quadratic equations fit to the log$_e$ of the dependent variables. Symbols on growth curves are predicted values from the regression model. The R^2 is coefficient of determination for the fit of the regression model. When less than five curves are shown, the missing curves closely overlap others: (1*b*) NF and NF × 1.4 = CF; (1*d*) NF and NF × 1.2 = CF; and (1*f*) NF and NF × 1.2 = CF.

highest O_3 concentrations may have caused a reduction (Fig. 2c and 2d). Plant height and the number of main-stem nodes were not affected by O_3, whereas water stress significantly reduced plant height but not the number of main-stem nodes (Table 1). Therefore, the primary effect of water stress on stems seemed to be to reduce internodal elongation. Total shoot dry wt. (all aboveground plant tissues) was reduced by O_3 ($p < 0.10$), although the effect was probably due to O_3 acceleration of leaf drop, for the most part.

Reproductive tissue dry wt. (squares, flowers, and bolls) was significantly affected by O_3 ($p < 0.10$) (Table 1). The growth curves indicate consistent reductions only in the two highest O_3 concentrations in the WW plots

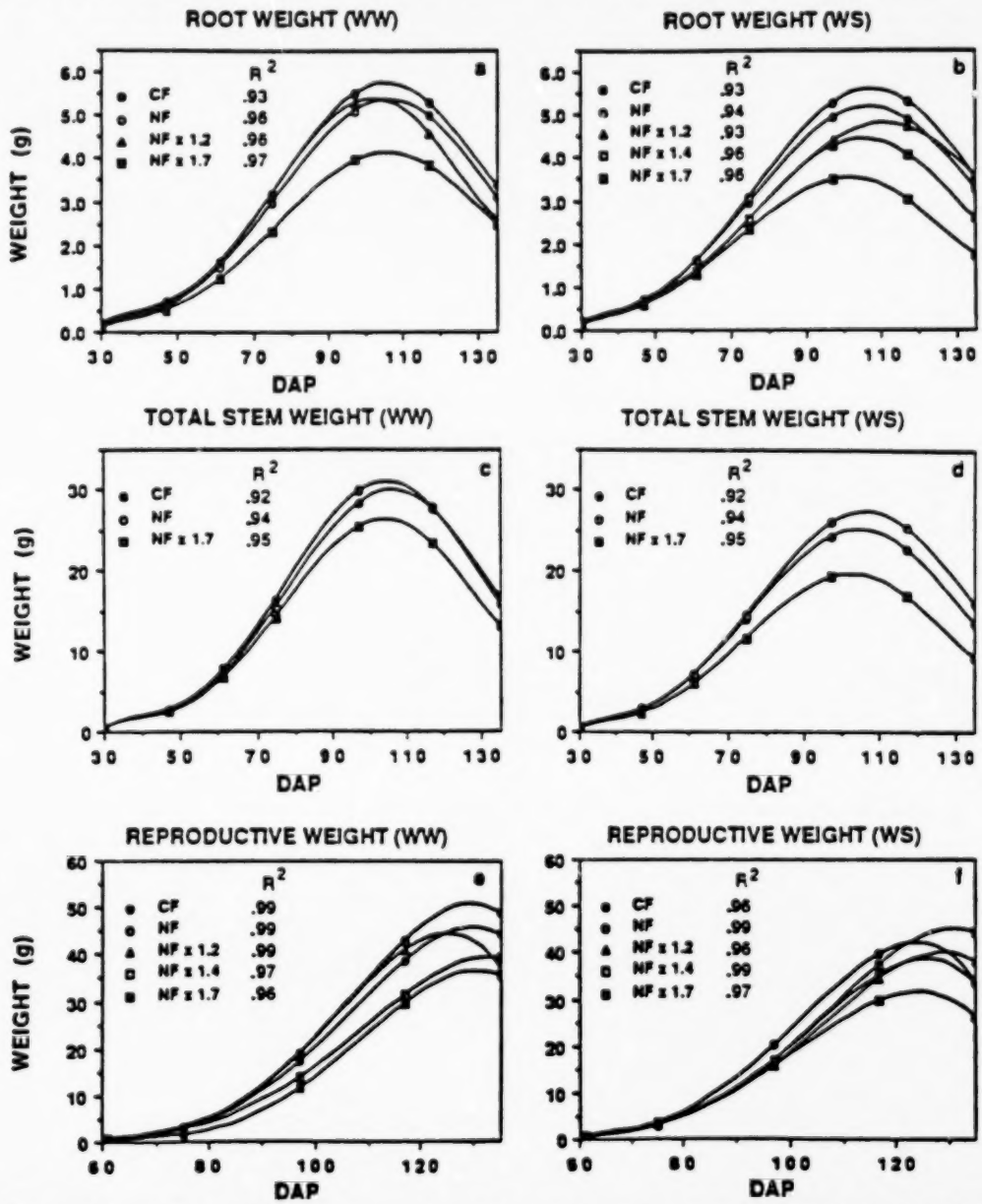

Fig. 2. Effects of chronic O_3 exposure on root dry wt. (*a* and *b*), total stem dry wt. (mainstem, branches, and petioles) (*c* and *d*), and reproductive tissue dry wt. (squares, flowers, and bolls) (*e* and *f*) per plant of McNair-235 cotton under well-watered (WW) and water-stressed (WS) conditions. Ozone exposures started on 30 DAP and continued through the last sampling date (135 DAP). Growth curves are quadratic equations fit to the log, of the dependent variables. Symbols on growth curves are predicted values from the regression model. The R^2 is coefficient of determination for the fit of the regression model. When less than five curves are shown, the missing curves closely overlap others: (2*a*) NF × 1.4 = NF × 1.7; (2*c*) NF × 1.2 = NF and NF × 1.4 = 1.7; and (2*d*) NF × 1.2 = NF and NF × 1.4 = CF.

and in the highest O_3 concentration in the WS plots (Fig. 2e and 2f). Boll dry wt. was significantly affected by O_3 and water stress (Table 1). Boll weights, however, were consistently reduced by O_3 only at the highest concentration in the WW plots.

Biomass Partitioning and Growth Analysis

The ratio of shoot to root biomass has been found to increase in response to O_3 stress in a number of studies with pot-grown plants in controlled environments (Kasana and Mansfield, 1986). Significant O_3 or water effects on shoot to root ratios were not found in the present study (Table 1). At the later sampling dates the ratios were affected by the acceleration of leaf drop by O_3, which tended to mask any effects on the relative development of shoot and root tissues. In order to obtain an expression that more nearly reflects the effects on partitioning of biomass between above- and belowground tissues, the ratio of total stem to root dry wt. (stem/root) was calculated (Fig. 3). This ratio was also affected by senescence late in the season, since O_3 accelerates abscission of petioles and stem tissue mass is lost as storage products are utilized for reproductive growth. Nevertheless, this ratio should more nearly reflect possible effects on the relative development of shoot and root tissues since the effects of leaf drop are removed. Ozone and water stress significantly affected the stem/root ratio ($p < 0.10$) (Table 1), with O_3 tending to increase the ratios and water stress reducing them (Fig. 3). The results were not consistent across all sampling dates, but the effects were most pronounced from 61 to 135 DAP. Trends for increased partitioning to aboveground tissues at the expense of roots have been seen in other experiments with pot-grown plants (Heck and Dunning, 1978; Oshima et al., 1978; Oshima et al., 1979; Flagler and Youngner, 1982; Blum et al., 1983). This has been ascribed to the utilization of energy for maintenance and repair of aboveground tissues at the expense of roots and a general priority of shoot growth over that of the root during periods of limited assimilate availability (Evans et al., 1964). A relative increase in shoot tissue at the expense of roots could have negative consequences in the event of severe drought conditions since the transpirational surface is increased while the water absorptive surface of the roots may become limiting.

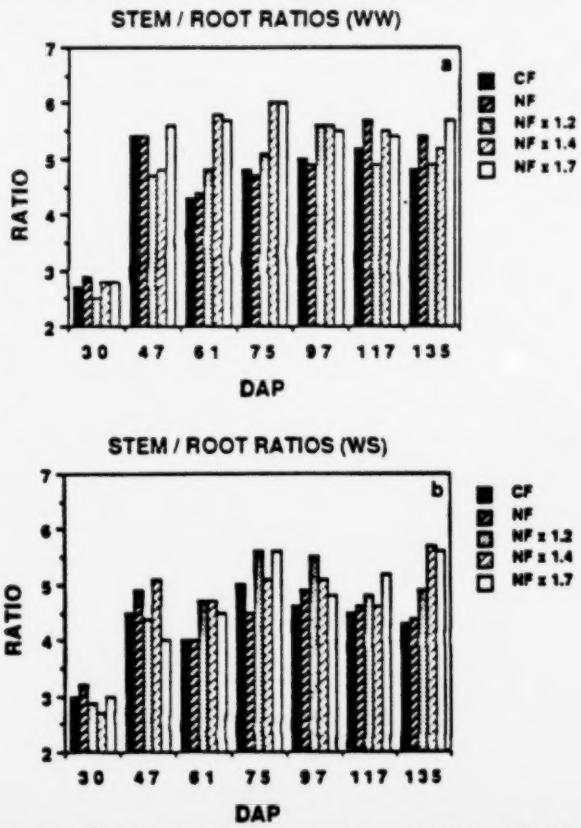

Fig. 3. Effects of chronic O_3 exposure on the ratio of total stem dry wt. (mainstem, branches, and petioles) to root dry wt. of McNair-235 cotton under well-watered (WW) and water-stressed (WS) conditions. Ozone exposures started on 30 DAP and continued through the last sampling date (135 DAP).

Since leaves were extensively affected by the O_3 treatments, growth analysis was applied to the leaf data according to procedures outlined by Hunt (1982). Specific leaf area (SLA), defined as square meter of leaf area per kilogram dry wt. of leaf, was significantly affected by O_3 and water stress (Table 1). Instantaneous SLAs, derived from the growth curves for leaf area and total leaf dry wt., were increased above the CF level by all O_3 treatments in the WW plots (Fig. 4a and 4b). This indicates that the mass of the leaves per unit leaf area was reduced by O_3. In the WS plots the effects were less evident. Water stress generally reduced the SLA throughout the season.

Leaf weight ratio (LWR), defined as the ratio of leaf dry wt. to total plant dry wt., was significantly affected by O_3 and water stress (Table 1). Instantaneous LWRs (derived from the growth curves for leaf dry wt. and total plant dry wt.) were slightly elevated by O_3 in the WW plots from approximately 40 to 80 DAP but this reversed starting at about 90 DAP (Fig. 4c and 4d). This indicates that O_3 stress increases the proportion of the total biomass in leaf tissue at the early growth stages, but leaf fall due to O_3 stress reverses this at the later stages. In the WS plots LWR was reduced late in the growing season due to O_3 stress. The water stress affect on LWR was most apparent late in the growing season in which case LWR was increased.

The instantaneous leaf area ratios (LAR), derived from leaf area and total plant dry wt., also illustrate the tendency of the plants to be relatively more "leafy" early in the growing season (40–80 DAP) in response to O_3 stress, especially in the WW plots (Fig. 4e and 4f). As with LWR, this reversed late in the season due to O_3-induced leaf drop.

The efficiency of the plants in producing total biomass as a function of leaf area is expressed as the net assimilation rate (NAR; g m^{-2} d^{-1}). In the WW plots, the NAR values were generally reduced by O_3 in all but the NF ×

1.2 treatment (Fig. 5). In the WS plots there was no obvious trend related to O_3 treatment.

Relative growth rates (RGR; kg kg^{-1} d^{-1}) were also calculated for total plant dry wt., but treatment related trends in plant RGR were not evident (data not shown). The RGR for the total plant is the product of NAR and LAR, and examination of these curves explains the lack of O_3-treatment effects on RGR. While NAR was generally reduced by O_3 during the phase of active growth in the WW plots, the LAR was increased. Thus, there appears to be a partial compensation, i.e., reduced photosynthetic efficiency due to O_3 was partially offset by greater relative leaf area. This is consistent with the results of Oshima et al. (1979) with greenhouse-grown

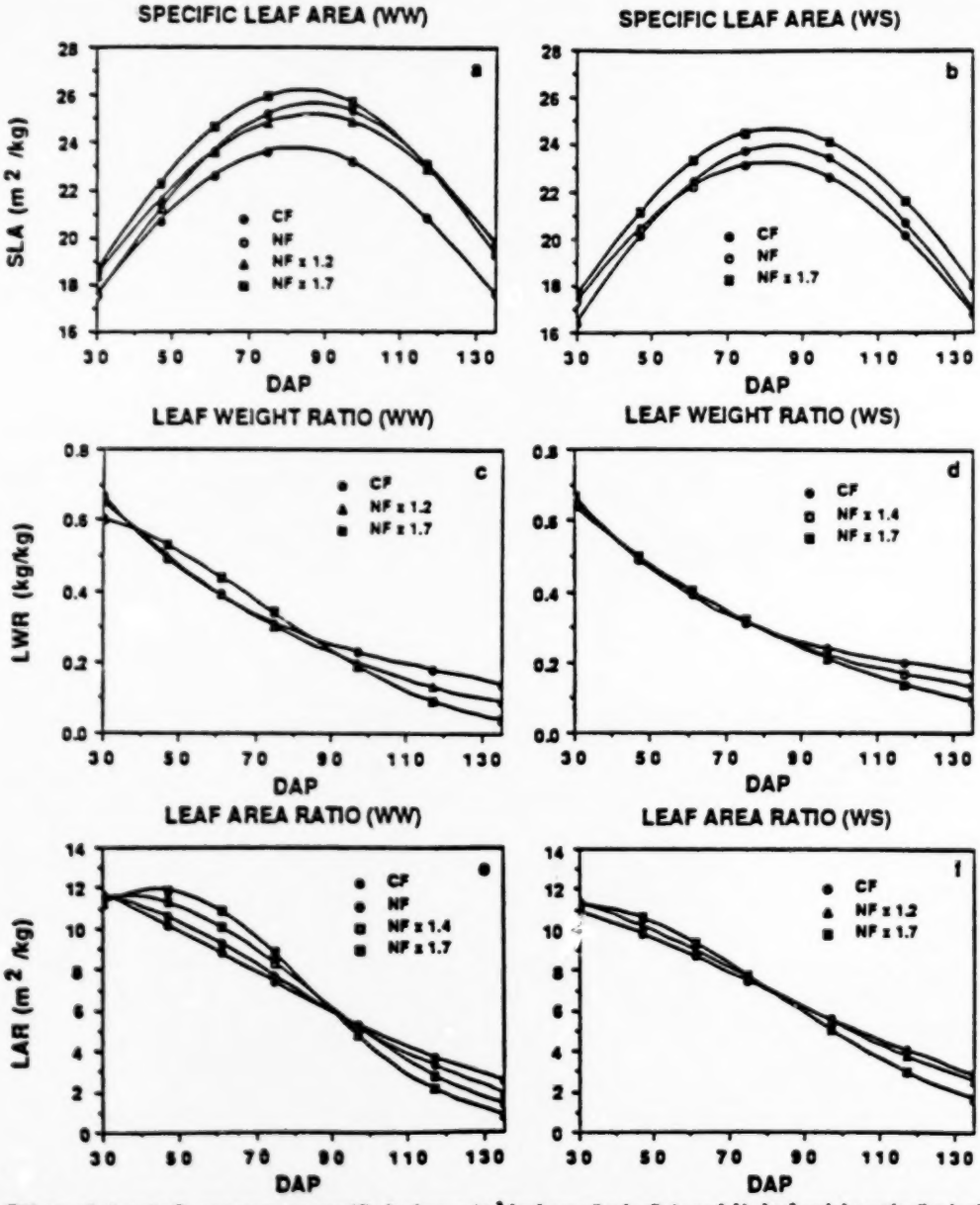

Fig. 4. Effects of chronic O_3 exposure on specific leaf area (m^2 leaf area/kg leaf) (*a* and *b*), leaf weight ratio (kg leaf/kg plant) (*c* and *d*), and leaf area ratio (m^2 leaf area/kg plant) (*e* and *f*) of McNair-235 cotton under well-watered (WW) and water-stressed (WS) conditions. Curves are instantaneous growth curves generated from $\log_e$-transformed dependent variables (Hunt, 1982). Symbols on growth curves are predicted values. Ozone exposures started on 30 DAP and continued through the last sampling date (135 DAP). When less than five curves are shown, the missing curves closely overlap others: (3*a*) NF × 1.4 = CF; (3*b*) NF × 1.2 = CF and NF × 1.4 = NF × 1.7; (3*c*) NF and NF × 1.4 = NF × 1.2; (3*d*) NF and NF × 1.2 = CF; (3*e*) NF × 1.2 = NF; and (3*f*) NF = CF and NF × 1.4 = NF × 1.7.

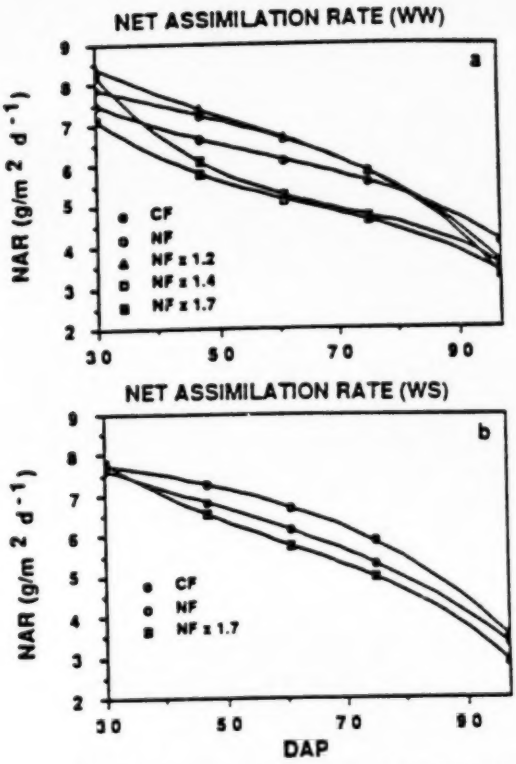

Fig. 5. Effects of chronic O_3 exposure on net assimilation rate (NAR) of McNair-235 cotton under well-watered (WW) and water-stressed (WS) conditions. Curves generated from log$_e$-transformed dependent variables (Hunt, 1982). Curves shown only through 97 DAP since values became very negative after that date. Symbols on growth curves are predicted values. Ozone exposures started on 30 DAP and continued throughout the last sampling date. When less than five curves are shown, the missing curves closely overlap others: (4*b*) NF × 1.2 and NF × 1.4 = CF.

cotton. While it is not possible to ascribe this response to specific biochemical or physiological mechanisms, it seems that under O_3 stress the plant is allocating more of its photosynthetic resources to the formation of new leaves. This has also been found with other plant species such as parsley [*Petroselinum crispum* (Mill.)] (Oshima et al., 1978).

Leaf area duration (LAD) has been shown to correlate with productivity and yield of plants in some cases (Watson, 1947). The LAD was calculated for each plot by integrating under the curves of leaf area over the course of the experimental period (30–135 DAP). Analysis of variance indicated that the O_3 treatments significantly affected LAD while the water treatments did not (Table 2). The LADs were progressively reduced as O_3 concentrations increased in the WW plots, while apparent reductions occurred in the WS plots only at the two highest levels. It can be seen that there is some correspondence between the LAD values from the present study and seed cotton yield obtained from contiguous rows in the same plots as reported by Heagle et al. (1988). The yield/LAD

Table 2. Ozone and water stress effects on leaf area duration (LAD), seed cotton yield, and the ratio of yield to LAD.

O_3 Treatment†	LAD‡		Seed cotton yield§		Yield/LAD	
	WW	WS	WW	WS	WW	WS
$\mu L\ L^{-1}$	$cm^2\ d\ (\times 10^5)$		$-g\ m^{-1}\ row-$		$- \times 10^{-3} -$	
0.022	2.35	2.15	443	373	1.71	1.59
0.041	2.18	2.14	424	349	1.77	1.49
0.051	2.05	2.15	355	371	1.57	1.58
0.061	1.97	1.99	320	339	1.49	1.54
0.073	1.68	1.63	234	300	1.27	1.68
	$P > F$					
Source						
O_3	0.047		0.011		0.65¶	
H_2O	0.767		0.663		0.88	
$O_3 \times H_2O$	0.93		0.324		0.19	

† Seasonal mean 12 h d^{-1} O_3 concentration.
‡ LAD calculated on leaf area basis (cm^2).
§ Yield results from Heagle et al. (1988).
¶ Ozone effect significant at $p < 0.043$ (linear) in WW plots when analyzed by regression analysis.

ratio was calculated in order to compare the correspondence between yield and LAD, and to express the relative efficiency of the leaves in producing seed cotton in the various treatments. The ratios are fairly constant across O_3 treatments for the WS plots, whereas in the WW plots the ratio seemingly declines at high O_3 concentrations. This decline in the ratio was significant in the WW plots ($p < 0.04$) when analyzed separately by ANOVA or regression analysis, indicating a decrease in the efficiency of the leaves in producing seed cotton. Unsworth et al. (1984) concluded that the efficiency of producing dry matter was a more important factor than leaf area in the O_3-induced reduction of soybean yield. The cotton leaf tissue, especially in the WW plots, showed symptoms of O_3 injury (mostly chlorosis) that generally corresponded to the level of the O_3 treatment (Heagle et al., 1988). Undoubtedly, the leaves were physiologically impaired as the injury symptoms developed, as also reflected in the reduction in NAR by O_3 in the WW plots (Fig. 5).

There is extensive literature indicating that O_3 stress reduces photosynthetic rates (e.g., Reich and Amundson, 1985; Amundson et al., 1987). There are also indications that translocation of photosynthetically assimilated C is impaired by O_3 (McLaughlin and McConathy, 1983), as well as the alteration of numerous other physiological processes such as carbohydrate metabolism (Koziol, 1984). Analysis of the soluble sugar and starch content of the tissues from this experiment illustrated that carbohydrate pools were affected by the O_3 treatments throughout the growing season (J.E. Miller, 1985, unpublished results). The lack of an effect on the yield/LAD ratio in the WS plots indicates that the efficiency of the leaves (throughout their lifespan on the plant) was not affected as severely by O_3 under water-stressed conditions. This is substantiated by the lesser amount of visible injury due to O_3 in the WS plots (Heagle et al., 1988). This may be due to a stomate-mediated reduction in gas exchange (O_3 uptake) under water-stressed conditions reducing the effective dose to the tissues, although one cannot rule out the possible contribution of a general

physiological hardening to other stresses as a result of water stress.

SUMMARY

Over the growing season, O_3 stress caused a depression of biomass of field-grown cotton, especially in leaf and root tissues. Stems seemed to be affected less by O_3. Water stress usually did not affect biomass of the tissues significantly, although the data suggest reductions, especially in maximum biomass attained at the lower O_3 concentrations. Significant interactions between O_3 and water stress were not found with any of the variables measured in this work. The physiological efficiency of the leaves was reduced in the WW plots as the O_3 concentrations increased as indicated by NAR values and a yield/LAD index. The data for partitioning of biomass between stem and root tissues, as well as the LWR and LAR results, indicate that allocation of C was modified by O_3. The results from this field study are generally consistent with previous work on effects of O_3 and water stress on plant growth and biomass partitioning from studies under controlled conditions. Nevertheless, additional research is needed to determine if principles concerning O_3 effects and interactions with environment, that have been established in controlled environments, apply under a variety of typical field conditions.

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